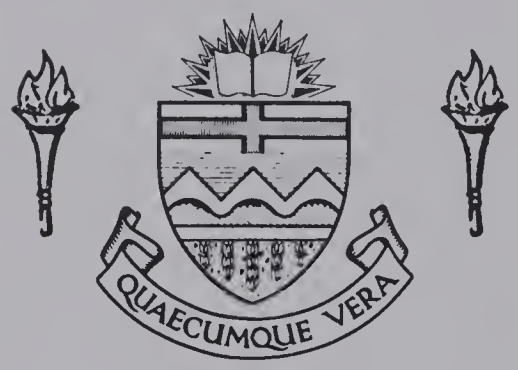

The University of Alberta
Printing Department
Edmonton, Alberta

THE UNIVERSITY OF ALBERTA

TRACE MINERAL STUDIES WITH FATTENING HOLSTEIN CALVES

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING, 1971

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Trace Mineral Studies with Fattening Holstein Calves" submitted by Michael Ivan, Ing. Agr., in partial fulfilment of the requirements for the degree of Master of Science.

Abstract

The effects of supplemental zinc, copper and manganese, alone or in combinations, in an all-barley ration were studied in sixteen Holstein-Friesian bull calves allotted by weight to eight treatments, with two calves per treatment. Feed consumption and growth rate were measured during a feeding trial of 10 weeks.

Metabolism studies were conducted for 12 days after the feeding trial was completed, and the calves were slaughtered. Apparent digestibility, retention of nitrogen and gross energy, and fecal and urinary excretion of zinc, copper and manganese were determined for each treatment. Blood samples were obtained from each calf twice daily for the last two days of the experiment.

When the calves were slaughtered the liver, heart and kidney, and samples of the contents from six segments of the gastrointestinal tract were taken for analyses of trace minerals.

Supplementation of the basal ration with zinc plus manganese resulted in slightly lower feed intake and rate of gain. Apparent digestion coefficients of dry matter, nitrogen and gross energy, and retention of nitrogen and gross energy were lower in this treatment suggesting a zinc-manganese interaction on feed utilization. Only the differences in digestion of nitrogen and gross energy, and retention of gross energy were significant ($P<0.05$).

Higher dietary manganese caused increased zinc concentrations in the liver ($P<0.05$), kidney ($P<0.05$), heart ($P<0.01$), and blood ($P<0.01$). The liver copper concentration was significantly decreased by dietary zinc ($P<0.05$) and increased by dietary copper ($P<0.01$) and manganese ($P<0.05$). Supplemental manganese increased ($P<0.05$) its

concentration in the liver.

Studies of absorption along the gastrointestinal tract indicated that net secretion of zinc appeared in the reticulo-rumen, small intestine and cecum, and net absorption in the omasum and abomasum. Net secretion of copper was found in the abomasum and net absorption from the rest of the tract, except that net secretion into the reticulo-rumen was associated with low dietary copper. Net secretion of manganese with all treatments was found only in the small intestine and cecum.

Fecal excretion of zinc was decreased ($P<0.01$) by its dietary supplementation. Higher dietary zinc and manganese increased ($P<0.01$), and copper decreased ($P<0.01$) fecal copper excretion. Fecal excretion of manganese was increased by its dietary supplementation but the differences were not significant ($P<0.05$).

Supplemental zinc decreased ($P<0.05$) the rate of its exretion in the urine. Urinary excretion of copper was decreased ($P<0.01$) by supplemental zinc and manganese. Higher dietary manganese decreased ($P<0.01$) the rate of its urinary excretion.

There was no evidence of deficiency of any trace minerals in the unsupplemented treatments. The addition of zinc, copper and manganese in excess to a ration composed primarily of barley did not improve the performance of bull calves.

Acknowledgements

The author wishes to thank Dr. L.W. McElroy, Chairman of the Department of Animal Science, for placing the facilities of the department at his disposal. The encouragement, direction and criticism given by Dr. C.M. Grieve, Associate Professor of Animal Nutrition, throughout the course of this study and in the preparation of the manuscript are greatly appreciated.

The assistance of Svend Nielsen, dairy herdsman at the Dairy Cattle Research Unit, the University of Alberta Edmonton Research Station and other members of the Research Unit staff is gratefully acknowledged.

The writer is also indebted to Dr. R.T. Hardin, Associate Professor of Poultry Genetics for assistance with statistical analysis of the data.

A research assistantship provided by the National Research Council is gratefully acknowledged.

Table of Contents

List of Tables

Page

Introduction

Until recently ruminants were fed diets comprised primarily of roughage and lesser amounts of concentrates. The diet varied within fairly narrow limits and the level of productivity of the ruminant was not particularly high.

In recent years there has been increasing demand for higher levels of production measured by growth rate, fattening or milk production, and more efficient utilization of the feed required for production. This has led to increased use of concentrates in place of roughage, and formulation of diets very different in composition to those formerly fed. Emphasis on automation has stimulated interest in these 'exotic' diets also, since there are inherent difficulties in automated feeding of roughage.

Higher levels of production and the use of high energy diets may increase the requirement for certain elements, whereas these diets may be lacking or deficient in some of the required elements, or certain elements may be present in undesirable proportions. Consequently, there is increased interest in supplements that will supply certain nutrients, such as trace minerals, that were previously adequate in most diets.

Trace minerals are known to be very important for biological functions in the animal body, since they are essential constituents of certain enzymes or are required for activity of the enzyme. Consequently, feed supplements are now available to supply the trace minerals zinc, copper, and manganese, which appear to be somewhat deficient in certain diets. However, it is also known that

imbalance of these trace minerals may result in problems that are more serious than a moderate deficiency. Therefore, it seemed desirable to carry out an experiment to study the effects of adding zinc, copper and manganese, alone and in combinations of these minerals, to high-grain diets fed to growing and fattening Holstein-Friesian calves. Measurements were made of feed consumption and growth rate of the calves, digestibility of the ration, absorption and excretion of the minerals, and the concentrations of these minerals in liver, kidney and heart tissue.

Review of Literature

Zinc

1. Historical

Todd et al. (1934) observed that growth rate and maximum weight attained were markedly reduced in rats fed a purified diet containing 1.6 parts per million (ppm) zinc, as compared with rats fed the same diet supplemented with zinc oxide to provide 5 ppm zinc.

The first acceptable explanation of a dietary requirement for zinc was presented by Keilin and Mann (1939), when they reported that a molecule of the enzyme carbonic anhydrase contained 0.33 percent zinc. Since then, zinc has been identified in other metallo-enzymes isolated from living tissue, including carboxypeptidase, alkaline phosphatase, alcohol dehydrogenase, glutamic dehydrogenase, and a number of pyridine nucleotide dehydrogenases (Underwood, 1966). Zinc has also been shown to act as a co-factor for many other enzymes.

Studies of zinc requirements by farm animals were stimulated by the discovery by Tucker and Salmon in 1955 (cited by Underwood, 1966) that additional zinc in the diet cured and prevented a condition in pigs known as parakeratosis. The condition was associated with excess dietary calcium.

2. Zinc Deficiency

Miller and Miller (1962) demonstrated zinc deficiency in calves by feeding a purified diet containing 2.7 ppm zinc. The calves developed parakeratosis, whereas calves fed a similar ration containing 45 ppm zinc did not develop abnormal symptoms. The symptoms of zinc deficiency that were observed included

anorexia, dull and listless appearance, reduced weight gains, alopecia, breaks in the skin with deep fissure formation around the hoofs, extensive dermatitis between the legs and behind the elbows, hard dehydrated skin on the body, inflammation of the nose and mouth with submucous hemorrhages, reduced blood levels of zinc, and reduced carbonic anhydrase activity. The addition of 260 ppm zinc to the diet resulted in a rapid dramatic recovery. Similar deficiency symptoms were observed by Ott et al. (1965) in calves fed a diet containing 3 ppm zinc. The symptoms were corrected within 3 to 4 weeks after the diet was supplemented with 100 ppm zinc, and serum levels of zinc increased from 18 to 116 mcg/100 ml of serum.

Symptoms of zinc deficiency have been observed in grazing cattle in Guyana and Finland. In other reports, zinc deficiency has been suspected in ruminants under practical field conditions, but insufficient information was available to confirm the diagonsis (Blackmon et al., 1967).

Zinc deficiency affects feed consumption and growth rate of ruminant animals. At the same level of feed intake, those fed a zinc-deficient diet grow at a slower rate and consume more feed per unit gain than those fed an adequate level of zinc (Blackmon et al., 1967; Miller et al., 1965 b). Miller et al. (1966 c) found that apparent digestibility of dry matter by calves was not affected by zinc deficiency. They suggested that lower feed efficiency attributed to zinc deficiency was the result of reduced utilization of the digested nutrients.

Animals do not have large reserves of zinc, since the capacity for storage in tissues other than bone is very limited. Consequently, as a zinc-deficient condition develops, there is usually a small decline

in the concentration of zinc in tissues such as liver, kidney, heart, bone and muscle, and a greater decline in the pancreas, which is the main organ for secretion of zinc (Underwood, 1966).

3. Zinc Toxicity

There appears to be a wide margin of safety between levels of zinc necessary for optimum growth and health, and those which might have deleterious or toxic effects on ruminant animals. Levels of 2000 ppm zinc have been used experimentally without any symptoms of toxicity (Miller et al., 1965 a).

4. Zinc Metabolism

Feaster et al. (1954) maintained steers for over a year on a diet containing 1000 ppm zinc, and found that most of the zinc was excreted. Approximately 70 percent of ^{65}Zn was excreted in feces and 0.3 percent in the urine. Retention of labelled zinc was highest in the soft tissues, such as pancreas, liver, pituitary, kidney and adrenal glands.

Miller et al. (1966 a) studied the physiological effects of zinc deficiency in calves and goats. Following single intravenous doses of ^{65}Zn the fecal excretion of ^{65}Zn was higher in normal animals fed a diet containing 46 ppm zinc than in comparable animals fed a zinc-deficient diet containing 6 ppm zinc. Specific activity of fecal zinc from animals fed the deficient diet was much higher, reflecting the much lower level of total fecal zinc. When fed the same diets, normal animals excreted more ^{65}Zn in the feces than did zinc-deficient animals. However, the deficient animals excreted more ^{65}Zn in the urine, suggesting a possible pathological effect of the deficiency on the kidneys. Levels of dietary zinc did not affect urinary excretion in normal animals. The results of

the experiment indicated that low levels of dietary zinc, and a condition of zinc deficiency, result in reduced endogenous fecal excretion of ^{65}Zn for at least two weeks after intravenous injection of zinc, thus contributing to homeostasis of this element.

Miller et al. (1968 b) showed that calves fed a purified diet low in zinc absorbed considerably more ^{65}Zn than those fed the diet with supplemental zinc, but there was no apparent difference in absorption or net retention of ^{65}Zn between calves fed a practical diet or a purified diet with supplemental zinc.

After oral dosing of calves with ^{65}Zn, the liver concentration of ^{65}Zn increased to maximum levels in 48 hours (Miller et al., 1967). The tissues of zinc deficient animals held ^{65}Zn more tenaciously than those of non-deficient calves. The zinc content of hair, blood, liver, lung, kidney, femur, skin and testicle was reduced, but in brain and muscle was not affected by a zinc-deficient diet (Miller et al., 1966 b), and there was little effect on dry matter content of the tissues. The concentration of zinc in blood plasma increased with increasing levels of dietary zinc (Miller et al., 1965a).

Absorption of zinc decreased with age in cattle (Miller and Cragle, 1965). Daily administration of ^{65}Zn resulted in absorption of 55 percent of the zinc by week-old calves, 20 percent by calves aged 5 to 12 months, and 12 percent by mature dairy cows. Absorption occurred in the abomasum and lower small intestine.

5. Zinc Requirement

The zinc requirement of cattle has not been fully determined. Studies have been conducted on the effects of supplemental zinc in practical rations, but a beneficial response was noted in only one experiment

(cited by Blackmon et al., 1967, and Thompson, 1970). Miller and Miller (1962) reported satisfactory weight gains and no deficiency symptoms in calves fed a diet containing 40 ppm zinc.

The composition of the diet has a marked effect on the amount of zinc required. The presence of chelating compounds, the levels of certain minerals such as calcium, and other unknown factors may increase the level of zinc required (Blackmon et al., 1967). Phytic acid reduces the availability of zinc to swine and poultry, but not to ruminants, because of the ability of the rumen microorganisms to break down phytates (Underwood, 1966).

Thompson (1970) reported that published data of zinc requirements have been in the range of 8.6 to 46, 45 to 72, and 83.5 to 100 ppm for calves and heifers, dairy cattle and beef cattle, respectively. He suggested that levels of 50, 60, and 90 ppm zinc be used for each group, respectively. The National Research Council (NRC) requirement for beef cattle (1970) is suggested to be between 10 and 30 ppm, whereas The Agricultural Research Council (ARC) requirement for ruminants (1965) is suggested to be 50 ppm.

Copper

1. Historical

The presence of copper was demonstrated in plant tissue in 1816 by Bucholz, and in animal tissue in 1833 by Boutigny (cited by Underwood, 1956). In 1928, Hart and co-workers (cited by Underwood, 1966), as a result of studies on milk anemia in rats, were the first to report that copper was an essential nutrient in animal diets. Some biochemical and physiological aspects of copper in animal nutrition were outlined by Cunningham (1931). A wider concept of the biological significance of the

element emerged from later studies that demonstrated the presence, in living cells, of copper-containing enzymes with oxidative functions, such as tyrosinase, uricase, ascorbic acid oxidase and cytochrome oxidase.

The first report of the natural occurrence of copper deficiency in cattle was in Florida (Neal et al., 1931), where a condition known as "Salt Sick" was found to respond to treatment with copper. Bennets and Chapman (1937, cited by Underwood, 1966), demonstrated that a demyelinating disease of lambs in Australia, named enzootic ataxia, could be prevented by administering copper to ewes during pregnancy.

The results of later research showed that the requirement for copper could be modified by other constituents of the diet (Dick and Bull, 1945, as cited by Dick, 1954). In Australia, chronic copper poisoning of sheep occurred when they grazed certain areas where the copper concentration in plants was within normal limits (Dick, 1954) Since then, extensive studies have been conducted to examine the interrelationships between copper and other minerals. The minerals found to have the greatest influence on copper metabolism have been molybdenum, phosphorus, sulphur, iron, zinc and cadmium (Thompson, 1970).

2. Copper Deficiency

"Salt Sick", a condition of cattle in Florida where growth rate was retarded by as much as one-half and reproductive performance was greatly impaired, was the source of considerable loss to the Florida cattle industry. Neal et al. (1931) demonstrated the condition was due to copper deficiency. Analyses of forage indicated deficient levels of iron and copper; supplementation with copper, but not with iron, was succesful in overcoming the problem.

The symptoms of copper deficiency are anemia, bone deformities,

neonatal ataxia, depigmentation of hair and wool, fibrosis of the myocardium, and scouring or diarrhoea (Underwood, 1966). The manifestations of copper deficiency depend on the animal species, the age and sex, the severity and duration of the deficient state, and on the particular environment in which the animal is maintained. As a result of inadequate intake and depletion of body reserves, the supply of copper becomes limited for the many metabolic processes requiring this element, and certain processes fail in the competition for the supply of copper.

The most striking effect that has been observed in copper-deficient animals, is a large reduction of their copper-containing enzymes, particularly cytochrome oxidase (Frieden, 1968). This enzyme is the principal "terminal" oxidase in all animals, or the last enzyme involved in oxidation of a substrate. When the final reaction cannot proceed, all the intermediate carriers remain in the reduced state and cannot be oxidized in the usual way. Since all energy in animals is derived from oxidative reactions, the terminal oxidase constitutes a crucial vulnerable point in metabolism.

Lamand et al. (1969) reported that bull calves, raised in a geographical area deficient in copper, developed growth disorders associated with severe cupremia. Four grams copper sulphate, incorporated with 200 grams of tallow, administered daily for 10 days caused a rapid and significant improvement in cupremia and growth rate. The inclusion of copper in the daily concentrate ration did not have as immediate an effect.

When copper and zinc levels in forage averaged below 5 and 40 ppm of the dry matter, respectively, the symptoms in cattle were general unthriftiness, reduced growth rate, fading of the skin around the eyes,

and sometimes a condition resembling parakeratosis of the skin on the bridge of the nose (Dynna and Havre, 1963). This complex copper-zinc deficiency sometimes responded to copper supplementation, but a mixture of copper and zinc, as sulphate or acetate, produced a remarkable response.

Extensive studies of the correlation between copper levels in blood and hair, and levels of different minerals in grass and soil were carried out by Binot et al. (1969) on 22 farms in Belgium. The results indicated that lead, zinc, calcium, sulphate and iron in grass, and lead, zinc, calcium, molybdenum, sulphate, manganese and pH in the soil were associated with deficiency symptoms in cattle. They concluded that cupremia in grazing cattle depended on the combination of conditions involving both grass and soil.

3. Copper Toxicity

Intake and digestion of copper in excess of the animal's requirement leads to accumulation of copper in the tissues, especially in the liver (Underwood, 1966). The liver of sheep and cattle accumulate large amounts of copper without apparent harmful effect, but beyond certain limits the liver suddenly liberates high levels of copper into the blood stream. Clinical symptoms may not appear for some time until hemolysis of the blood cells occurs followed by sudden death.

Calves fed a milk substitute containing 115 ppm copper developed typical signs of chronic copper poisoning, including hemoglobinaemia, hemoglobinuria and jaundice (Shad and Lewis, 1957). Weiss and Baur (1968) observed that calves fed high levels of copper died 1 to 2 days after symptoms of copper toxicity were observed. During the hemolytic crisis, there were marked increases in serum levels of copper, enzymes

and iron, up to 60 percent of the total hemoglobin was in the form of methemoglobin, and liver concentrations of copper ranged between 898 and 2091 ppm of the dry matter.

Todd and Thompson (1965) fed diets containing 500 ppm copper to calves until the calves died after 20 to 21 weeks. They observed that blood levels of copper increased 5 to 10 times the normal level during the hemolytic crisis, and concentrations of reduced and oxidized glutathione were lowered. One week before the hemolytic crisis there was an increase in the activities of lactic dehydrogenase and glutamic-oxaloacetic transaminase in blood plasma.

4. Copper Metabolism

The liver is the main organ for storage of copper in the body, so concentrations of copper in the liver can be used as an index of the copper status of the animal. The experiments reported by Dick (1954) showed that increased copper intake by sheep was reflected in increased copper content of the liver. The amount of copper accumulated was proportional to the intake of copper within the range of 3 to 20 mg daily. Over a period of 6 months, storage in the liver amounted to 4.5 to 5.0 percent of the copper consumed. Gartner et al. (1968) noted that levels of copper in the liver increased after subcutaneous injection of steers with copper glycinate. It has also been observed that calves, from cows treated with copper, had higher levels of copper in liver and blood, than did calves from cows that were not treated (Alexander et al., 1967; Hewetson, 1963).

The magnitude of the increase in liver concentrations of copper depends on a number of factors. The addition of ferrous sulphide to the diet lowered the expected copper accumulation in the liver by 75 percent

(Dick, 1954), presumably by conversion of copper to the insoluble sulphide form. Molybdenum was also found to severely limit the accumulation of copper, but only when the diet contained sufficient quantities of inorganic sulphate. Similar results were obtained by Vanderveen and Keener (1964). Cobalt therapy decreased liver levels of copper in grazing steers (Gartner et al., 1968), and silver had a similar effect in rats (Van Campen, 1966), but also increased the relative proportions of copper in the heart and spleen. Dempsey et al. (1958) concluded that the concentration of copper in liver and blood serum of rats was markedly influenced by the intake of copper in the diet, and that there was a high correlation between serum and liver copper concentrations.

Copper concentrations in livers of rats fed high levels of copper were influenced by zinc and protein (McCall and Davis, 1961). It was a complex interaction dependent on the relative concentration of each of the factors present. Protein appeared to have an effect on regulation of copper by increasing elimination of copper from the liver when it approached toxic levels. High zinc decreased liver copper concentrations, but had no effect when a high level of protein was included in the diet.

The percentage of copper uptake by the liver is distinctly lower in the last 6 months of pregnancy than in the first 3 months or than in non-pregnant cows (Binnerts, 1967). This confirms the hypothesis that the fetal liver derives its copper mainly from the maternal blood supply of inorganic copper, rather than from the ceruloplasmin.

Dube (1967) reported that high levels of calcium in rations fed to calves caused a significant rise in serum calcium levels and a decline in serum copper levels. Urinary excretion of copper was increased by one-third, and retention of copper was reduced by a high intake of calcium.

Bosman (1964) found that 48 percent of the total copper in grass was soluble in 0.1 N acetic acid, but that copper in rumen contents was converted to a less soluble form, possibly copper sulphide. This is in agreement with the supposition that a high proportion of copper in the rumen is present as copper sulphide.

5. Copper Requirement

Many factors influence copper metabolism and increase or decrease the animal's requirement for this element. Consequently, it is difficult to suggest an optimum level of copper in the diet that will provide maximum performance of the animal.

Dowdy and Matrone (1968) showed that sheep appeared normal when fed a diet containing a low level of copper, but developed anemia when molybdenum was added to the diet. Many other factors such as cobalt (Gartner et al., 1968), ferrous sulphide (Dick, 1954), zinc (Magee and Matrone, 1960), calcium (Dube, 1967), protein (Reinhold et al., 1967), cadmium and silver (Van Campen, 1966) affect deposition or excretion of copper from the body.

Data that have been published (Thompson, 1970) indicate copper requirements range from 4.8 to 6.9 ppm of the diet. He suggested a practical level of 7 ppm for all cattle. The NRC (1970) requirements list 4 ppm when the diet contains low levels of molybdenum and sulphate, but a 2- to 3-fold increase when levels of molybdenum and sulphate are high. The ARC (1965) requirements suggest 10 ppm for cattle and 5 ppm for sheep.

Zinc-Copper Interrelationships

In 1937, Sutton and Nelson (cited by Smith and Larson, 1946) observed that excess dietary zinc produced anemia, subnormal growth and

reproductive failure in rats, and the anemia was completely prevented by feeding iron, copper and cobalt salts. Smith and Larson (1946) obtained similar results and showed that feeding supplemental copper maintained a high level of hemoglobin.

In studies of copper toxicity at the University of Florida (Davis, 1958), copper was demonstrated to have a marked inverse relationship to zinc, at least within the livers of animals. When levels of copper in the liver rose to values above 3000 ppm, zinc levels declined from normal values of 300 ppm to a level of approximately 1 ppm. Conversely, increasing the level of dietary zinc caused a depression in the level of copper in the liver, but only when dietary levels of copper were in the borderline to normal range of 5 to 10 ppm.

Magee and Matrone (1960) indicated that zinc interfered with copper metabolism in the rat by decreasing utilization and increasing excretion of copper, but had no apparent effect on absorption of copper. Cox and Harris (1960) showed that accumulation of zinc in the liver caused a marked loss of iron, resulting in the production of anemia and depression of the activity of some iron-containing enzymes. They concluded that copper increased mobilization of iron to counteract the anemia and reduction in enzyme activity.

Ritchie et al. (1963) indicated that the addition of 100 ppm zinc to a diet containing 250 ppm copper counteracted copper toxicity that had developed, and reduced liver levels of copper in the rat. McCall and Davis (1961) observed a similar effect of zinc on liver levels of copper in the rat.

Copper and zinc induce a competitive mechanism within the foetal and placental structures of the rat, resulting in lower uptake of

radioactive zinc in those tissues (Kinnamon, 1963).

Starcher (1969), in studies of copper absorption in the chick, observed ^{64}Cu in the duodenal mucosa was firmly and specifically attached to proteins with a molecular weight of approximately 10,000. Zinc appeared to act as an inhibitor of copper absorption by binding to, and displacing copper from, the duodenal protein.

Manganese

1. Historical

Manganese has been recognized for half of this century as a constant constituent of plant and animal tissue. Early studies by McHargue (1926) showed that compounds of manganese had important biological functions in animal metabolism.

In 1931, Kemmerrer et al., Vaddell et al., and Orent and McCollum (cited by Underwood, 1966) reported independently that manganese was necessary for growth and fertility in mice and rats. A few years later, two diseases of poultry, known as perosis and nutritional chondrodystrophy, were found to respond to supplemental manganese (Wilgus et al., 1936).

These discoveries stimulated further studies of the distribution of manganese and of its requirements and mode of action in the animal body.

2. Manganese Deficiency and Requirement

Manganese deficiency is extremly rare in cattle under natural conditions of grazing or stall feeding, because most common feeds normally contain high levels of manganese. Most pasture and hay crops contain 50 to 150 ppm manganese on the basis of dry weight, and seeds with the exception of corn contain 15 to 50 ppm manganese (Underwood,

1956).

Bentley and Philips (1951) found that 10 ppm manganese in the diet appeared to be a minimum or borderline requirement for cattle. They suggested practical diets should be supplemented if the concentration of manganese was below 20 ppm. Diets containing less than 10 ppm manganese were adequate for growth of heifers, but resulted in delayed sexual maturity and pregnancy, lower concentrations of manganese in the ovaries, and abnormal structural changes in the liver. Their calves tended to have weak legs.

Dyer et al. (1964) reported 16 ppm manganese in the diet was not adequate for heifers in gestation, since they gave birth to calves with slight leg deformities, reduced serum alkaline phosphatase, and reduced concentrations of manganese in bone and liver. Diets containing 56 ppm manganese resulted in the birth of normal calves. Rojas et al. (1965) also suggested the requirement for manganese was in excess of 16 ppm in the diet and recommended at least 20 ppm on the basis of concentrations in the livers of normal and deficient calves.

There is evidence of naturally-occurring manganese deficiency in cattle grazing certain farms in Holland, where sandy and peat soils with high pH are found. The manganese content of the forage appears normal (44 to 199 ppm), but manganese supplementation prevents or cures the symptons of retarded growth and developemnt of young cattle, leg deformities of calves, and low fertility of cows (Underwood, 1966).

Some evidence suggests that deficiency of manganese in the diet results in more efficient absorption of manganese, and that excess calcium and phosphorus reduce the availability and increase the dietary requirement of manganese (Thompson, 1970).

Thompson (1970) suggested levels of 30 ppm manganese for calves and heifers, and 20 ppm for older cattle as dietary requirements. The NRC (1970) requirement indicates supplementation with manganese is required only in all-concentrate diets based on corn. The ARC (1965) suggest a dietary concentration of 40 ppm of the dry matter.

3. Manganese Metabolism

The liver appears to be actively involved in manganese metabolism. Concentrations of the element in the liver vary with age and species of animal, but the variation is small by comparison with that of other trace minerals (Lorenzen and Smith, 1947).

Gessert et al. (1952) noted that supplemental manganese in the diet of cows tended to increase liver concentrations of manganese. Lassiter and Morton (1968) obtained similar results with lambs, and also noted increased manganese concentrations in heart tissue, but not in kidney or muscle tissue.

Manganese resembles iron and zinc to the extent that it is poorly absorbed and is excreted largely in the feces. The kidney does not appear to function actively in elimination of manganese, and very little appears in the urine (Underwood, 1956).

Greenberg et al. (1943) observed that oral administration of manganese resulted in the appearance of about 1 percent of the dose in bile, whereas injection of manganese resulted in the appearance of 25 to 35 percent of the dose in bile. They suggested that manganese, in excess of tissue requirements, was carried to the liver prior to its excretion in the bile.

Cotzias (1960) indicated that interactions existed between manganese and other minerals, but that these probably occurred outside of the body and were not metabolic interactions. Within

the body, manganese was transported by a specific protein, transmanganin, in the plasma, and other segments of its pathway through the body also appeared to be specific for manganese. Diez-Ewald et al. (1968) reported that increased iron absorption resulted in increased absorption of manganese.

Most of the research on manganese in nutrition has been carried out with rats and other laboratory animals. Very little research has been conducted with ruminants because there have been few problems associated with manganese in ruminant nutrition.

Experiments at the University of Alberta

Introduction

Trace minerals are usually added to cattle rations to prevent or overcome deficiencies and possible consequences on animal performance. The need for trace mineral supplementation has not been defined because of wide variation between feeds in their mineral content. This variation is due, in part at least, to the amount of the elements in the soil, availabilty of the soil minerals to the plants grown, and availability of the plant minerals to the animals fed.

It has been indicated that deficiencies of zinc (Blackmon et al., 1967; Miller et al., 1965 b), copper (Lamand et al., 1969; Neal et al., 1931) and manganese (Bentley and Philips, 1951) resulted in decreased growth rate, which was improved by addition of the deficient mineral to the diet. Studies on the effect of individual trace minerals on a normal performance are of little value, because of interactions between the minerals.

The main objective of the present experiment was to study the effects of the addition of three trace minerals (zinc, copper and manganese) to a practical all-concentrate diet on the performance of bull calves. A second objective was to study interactions among these trace minerals.

Consequently, measurements were made of growth rate, feed consumption, efficiency of feed utilization, digestibility of dry matter, nitrogen and gross energy, absorption and excretion of zinc, copper and manganese, and the concentrations of the minerals in the liver, kidney and heart.

Experimental

Animals

Sixteen Holstein-Friesian bull calves from the Dairy Cattle Research unit, University of Alberta Edmonton Research Station were allotted by weight to eight treatments, with two calves per treatment.

The eight treatments were as follows:

Treatment 1 - basal ration

Treatment 2 - basal + Zn

Treatment 3 - basal + Cu

Treatment 4 - basal + Zn + Cu

Treatment 5 - basal + Mn

Treatment 6 - basal + Zn + Mn

Treatment 7 - basal + Cu + Mn

Treatment 8 - basal + Zn + Cu + Mn

The experiment was carried out as animals and facilities became available between June, 1969 and February, 1970. Treatments 2, 3 and 4 were completed before 5, 6, 7 and 8 commenced; Treatment 1 was carried out in both periods, using one calf in each period. The calves in each treatment varied in average initial age from 22 to 28 weeks and in average initial liveweight from 111 to 168 kg.

The calves were kept in individual stalls and were full-fed the experimental diets twice daily. Water and a mixture of equal parts by weight calcium phosphate and cobaltized-iodized salt were available free choice.

Initial liveweights were obtained from two successive weighings at 24 hour intervals after the calves were without feed and water overnight. After a feeding period of 10 weeks, the calves were weighed

again after being without feed and water overnight. Metabolism studies were carried out for an additional 12 days, before the calves were slaughtered.

Experimental Rations

The basal ration (Table 1) was formulated for all eight treatments, and consisted primarily of barley. Urea was included to provide a calculated level of 12 percent protein equivalent.

The experimental rations (Table 2) were formulated by the addition of salts of the particular trace minerals to the basal ration. The amount of the trace minerals to be added was calculated from the analysis of the barley, which contained 40, 6 and 12 ppm of Zn, Cu and Mn, respectively.

All supplements to each ration were mixed with 9 percent of the rolled barley in a Hobart mixer, and each ration was then prepared by adding the correct premix to the remaining 92 percent rolled barley.

Metabolism Studies

Metabolism studies were conducted after the 10-week feeding period was completed. Chromic oxide (Cr_2O_3) was used as an indicator of fecal excretion (Maynard and Loosli, 1962). 0.5 percent of Cr_2O_3 was mixed into each ration fed to the experimental animals. To ensure complete consumption of the daily ration, the feed offered was restricted by 0.5 kg below the average daily consumption for each calf during the final week of the feeding period. The daily rations were divided into two parts and fed at 9 A.M. and 5 P.M. for 12 days.

Feed samples were taken twice daily for each ration under test. The samples for each ration were combined, ground in a

Table 1

Formulation of the basal ration.

Ingredients	%
Barley	97.6
Urea	0.8
Limestone	1.0
Cobaltized-Iodized salt	0.5
Vitamin A (10,000 IU/gm.)	0.088
Vitamin D (35,000 IU/gm.)	0.0055
Vitamin E (44 IU/gm.)	0.015
Total	100.0

Chemical analysis	
Dry matter, %	88.1
Crude protein (air dry basis), %	12.6
Gross energy (air dry basis), Mcal/kg	4.12
Ca, %	0.46
P, %	0.40

laboratory mill and stored for further chemical analysis.

Fecal grab-samples were obtained at 6 A.M., 12 A.M., 5 P.M. and 11 P.M. daily, on days 7 to 12 inclusive, of the metabolism trial. The samples were immediately frozen, and later were dried in a forced-draft oven for 48 hours and ground in a laboratory mill. Ten grams were taken from each ground grab-sample and composited to

Table 2

Formulation of the experimental rations.

Treatment ⟶	1	2	3	4	5	6	7	8
Ration ⟶	Control	Zn	Cu	Zn+Cu	Mn	Zn+Mn	Cu+Mn	Zn+Cu+Mn
Ingredients								
Basal ration, kg	100	100	100	100	100	100	100	100
$ZnSO_4.7H_2O$, gm.		26.5		26.5		26.5		26.5
$CuSO_4.5H_2O$, gm.			5.7	5.7			5.7	5.7
$MnSO_4.H_2O$, gm.					11.9	11.9	11.9	11.9
Analysis								
Zn, ppm	40	100	40	101	40	102	40	97
Cu, ppm	6	6	20	21	6	6	21	19
Mn, ppm	12	12	12	12	50	51	49	47

provide one fecal sample per calf. The composite samples were stored for chemical analysis.

Total urine with 15 ml of 50% (v/v) H_2SO_4 was collected from each animal at 9 A.M. and 5 P.M. daily for 48 hours. Five percent by volume was transferred to polyethylene bottles and stored at 4°C for further chemical analysis.

Blood Samples

Blood samples were obtained from each calf at 9 A.M. and 5 P.M. daily for the last two days before the calves were slaughtered. Vacuum tubes with heparin were used to obtain approximately 15 ml of blood from the jugular vein. The samples were stored at -18°C for subsequent analysis for trace minerals.

Liver, Kidney and Heart Samples

When the calves were slaughtered, the heart, right kidney and samples of the liver from each calf were taken. These samples were weighed and dried in a freeze-drier for three days. After weighing to determine dry matter, the samples were homogenized in a laboratory blender and stored for analysis of trace minerals.

Gastrointestinal Tract Samples

On the morning after the metabolism studies were completed, the calves were fed one-half of the daily ration containing 0.5 percent of Cr_2O_3. Approximately 5 hours later the calves were slaughtered. The gastrointestinal tract of each calf was tied off, removed, and divided into the:

reticulo-rumen

omasum

abomasum

small intestine

cecum

large intestine

The contents of each segment were weighed and mixed, and a portion was dried at 70°C for five days. Dry samples were ground in a laboratory mill and stored for analysis.

Chemical Analyses

The methods of AOAC (1965) were used for determinations of dry matter in the feed, fecal and gastrointestinal tract samples, and of nitrogen in the feed, fecal and urine samples. The dry matter of urine was determined by freeze-drying a 5-ml sample of the urine for 48 hours. Freeze-drying was also used for determination of the dry matter of the liver, kidney and heart tissues.

Gross energy in feed and fecal samples, and in freeze-dried urine samples was measured by combustion in a Parr oxygen bomb calorimeter.

Determination of Cr_2O_3 in the feed, fecal and gastrointestinal samples was according to the method of Hill and Anderson (1958).

Atomic Absorption Spectrophotometry (Techtron AA-3 with computer) was used for analyses of Cu, Zn and Mn. The dried samples of feed, feces, urine, gastrointestinal tract, liver, kidney and heart were prepared by wet ashing as outlined in AOAC (1965). Blood samples were prepared by the method of Olson and Hamlin (1968). Fisher's Certified Atomic Absorption Standards were used to prepare working standards of each mineral, ranging from 1 to 20 ppm. The quantity of

mineral in the sample was determined by comparing its absorption with the absorption curve of the corresponding standard.

Apparent net trace mineral absorption in different segments of the gastrointestinal tract was calculated for each segment using the equation reported by Miller (1967), except that ppm was used in place of percentage, as follows:

$$\% \text{ absorption} = 100 - 100 \left(\frac{\text{ppm } Cr_2O_3 \text{ in feed}}{\text{ppm } Cr_2O_3 \text{ in ingesta}} \times \frac{\text{ppm mineral in ingesta}}{\text{ppm mineral in feed}} \right).$$

The results were expressed in relation to the daily intake of the trace mineral, that is, net absorption that had taken place up to that particular segment of the gastrointestinal tract (Miller and Cragle, 1965). The results were also expressed in relation to the preceding segment of the tract (Yang and Thomas, 1965). Negative absorption values indicate net secretion.

Statistical Analyses

Calculations and statistical analyses of the data were performed on the IBM 360/67 computer in the University of Alberta Computing Center. Analysis of variance computer program written by Smillie (1969) were used for analysis. F tables in Steel and Torrie (1960) were used to determine the level of significance.

The main effects of each mineral, averaged over the combination of the other minerals are presented.

Standard errors were calculated for these factorial means.

Results and Discussion

Average Daily Feed, Daily Gain, and Feed Conversion

There were differences between treatments in average initial and final liveweight (Table 3). Consequently, to reduce variability in feed consumption associated with differences in liveweight, average daily feed (ADF) consumption was calculated on the basis of metabolic weight (MW) as follows:

$$\text{ADF/100 units MW} = \frac{\text{average daily feed (kg)}}{\left(\frac{\text{initial + final liveweight}}{2}\right)0.75} \times 100.$$

Average daily feed consumption ranged from 9.2 to 11.1 kg per 100 units of metabolic weight, and there were no significant differences ($P<0.05$) between any of the treatments (Table 3). When the factorial means were calculated, there were no significant differences ($P<0.05$) detected (Table 3), although copper appeared to increase feed intake by 4 percent and manganese to decrease it by 8 percent.

Supplementation with zinc and copper, alone or in combination with each other, resulted in slightly faster gains than obtained with the ration containing no trace mineral supplements (Table 3). Supplementation with manganese resulted in lower gains than obtained with rations not containing supplemental manganese. When the factorial means were calculated (Table 3), it appeared that zinc had no effect on growth rate, copper increased it by 5 percent, and manganese decreased it by 17 percent. However, none of the differences was significant ($P<0.05$). The overall average daily gain was 1.42 kg, as compared with the average of 1.2 kg obtained by Kehoe (1969). Miller and Miller (1962) reported satisfactory weight gains when the ration contained 40 ppm of zinc, which agrees with data in this experiment.

Table 3

Average daily feed consumption, daily gain and feed conversion

Treatment	Initial live-weight	Final live-weight	Daily feed intake	ADF/100 units MW	Gain per day	Feed per kg gain
	kg	kg	kg	kg	kg	kg
1. Control	147	248	5.1	9.6	1.44	3.5
2. Zn	164	277	6.2	10.9	1.62	3.9
3. Cu	163	274	6.3	11.1	1.59	4.1
4. Zn + Cu	168	277	6.1	10.6	1.55	3.9
5. Mn	111	202	4.4	9.9	1.29	3.4
6. Zn + Mn	137	218	4.5	9.2	1.16	3.9
7. Cu + Mn	121	215	4.5	9.7	1.34	3.3
8. Zn + Cu + Mn	130	221	4.7	10.0	1.31	3.6
Factor Level						
Zn Unsupplemented				10.1	1.42	3.6
Supplemented				10.1	1.41	3.8
Cu Unsupplemented				9.9	1.38	3.6
Supplemented				10.3	1.45	3.7
Mn Unsupplemented				10.5	1.55	3.8
Supplemented				9.7	1.28	3.5
Standard error				0.4	0.09	0.1

There were no significant differences ($P<0.05$) in feed consumed per unit gain (Table 3), and the values ranged from 3.3 to 4.1 kg feed per kg gain. It would be expected that deficiences of zinc and copper would result in poorer feed efficiency (Blackmon et al., 1967; Lamand et al., 1969; Miller et al., 1965 b;, Neal et al., 1931). Consequently, it would appear that none of the rations in this experiment were deficient in these minerals.

Thompson (1970) recommended levels of 50, 7 and 30 ppm of zinc, copper and manganese, respectively in practical rations for cattle. The ARC (1965) requirements suggest levels of 50, 10 and 40 ppm of zinc, copper and manganese, respectively. The rations in this experiment contained 40, 6 and 12 ppm of zinc, copper and manganese, respectively, before supplementation with any of these minerals. These levels appeared adequate for satisfactory performance of the calves, since addition of the trace minerals did not improve their performance.

Apparent Digestibility of Dry Matter,
Nitrogen and Gross Energy

There were no appreciable differences between treatments in the apparent digestibility of dry matter (Table 4), although the ration containing zinc plus manganese (Treatment 6) had a lower coefficient than did the other rations. When the factorial means were calculated (Table 4), the addition of zinc, copper or manganese had no significant effect ($P<0.05$) on the digestion coefficients of dry matter. The lack of effect of zinc on digestibility of dry matter agrees with results obtained by Miller et al. (1966 c).

The apparent digestion coefficients of nitrogen and gross energy were significantly lower ($P<0.05$) in the ration with zinc and manganese

Table 4

Apparent digestion coefficients.

Treatment		Dry matter	Nitrogen	Gross energy
		%	%	%
1.	Control	81.5	80.7	81.2
2.	Zn	83.4	82.8	82.8
3.	Cu	82.6	80.5	81.1
4.	Zn + Cu	84.2	82.0	84.1
5.	Mn	84.1	84.0	85.7
6.	Zn + Mn	78.3	75.8	79.5
7.	Cu + Mn	83.7	81.9	85.7
8.	Zn + Cu + Mn	82.0	78.2	83.1
Overall average		82.5	80.7	82.9
Factor	Level			
Zn	Unsupplemented	82.9	81.8	83.4
	Supplemented	82.0	79.7	82.4
Cu	Unsupplemented	81.8	80.8	82.3
	Supplemented	83.1	80.7	83.5
Mn	Unsupplemented	82.9	81.5	82.3
	Supplemented	82.0	80.0	83.5
Standard error		0.9	1.1	0.9

than in the other rations (Table 4). This could be partly attributed to the lower digestibility of dry matter in this ration, which would result in a greater fecal excretion of nitrogen and gross energy. When the factorial means were calculated (Table 4), there were no differences between digestion coefficients that could be attributed to the addition of the trace minerals.

Nitrogen Retention

It appeared that calves in Treatments 1 to 4 retained more total nitrogen daily than those in Treatments 5 to 8 (Table 5). However, they were larger calves and consumed more feed and nitrogen per day. When percentage retention was calculated (Table 5), there were no appreciable differences. The values for percentage retention agree fairlywell with those obtained by Kehoe (1969). When the factorial means were calculated (Table 5), there were no significant effects ($P < 0.05$) attributed to the addition of the trace minerals to the rations. The results indicate that nitrogen retention was not affected by the addition of zinc, copper, manganese, or combinations of these minerals to all-barley rations.

Average Daily Energy Retention

It appeared that calves in Treatments 5 to 8 retained less energy daily than those in Treatments 1 to 4 (Table 6), when fecal and urinary losses were deducted from the amounts consumed. However, the calves in the last four treatments were smaller and consumed less feed and less energy daily than those in Treatments 1 to 4. When percentage retention was calculated (Table 6) there were no apparent differences except for a lower retention by calves fed the ration containing zinc and manganese (Treatment 6). This was associated with slightly lower feed intake and

Table 5

Average daily nitrogen retention.

Treatment	Nitrogen in feed	Nitrogen in feces	Nitrogen in urine	Nitrogen retained	Nitrogen retained
	g	g	g	g	%
1. Control	107.0	20.0	42.1	44.9	42.0
2. Zn	119.1	20.6	49.2	49.3	41.4
3. Cu	128.7	25.2	47.9	55.6	43.2
4. Zn+Cu	126.9	22.9	44.4	59.6	47.0
5. Mn	84.4	13.5	31.7	39.2	46.4
6. Zn+Mn	80.5	19.5	35.0	26.0	32.3
7. Cu+Mn	81.3	14.7	35.6	31.0	38.1
8. Zn+Cu+Mn	79.4	17.3	34.9	27.2	34.3
Overall average					40.6
Factor Level					
Zn Unsupplemented					42.4
Zn Supplemented					38.5
Cu Unsupplemented					40.2
Cu Supplemented					40.6
Mn Unsupplemented					43.1
Mn Supplemented					37.7
Standard error					3.4

digestibility of energy by calves in Treatment 6.

No significant differences were detected ($P<0.05$) when the factorial means were calculated (Table 6). This indicates that the addition of zinc, copper and manganese to high-barley rations had no appreciable effect on energy intake, or fecal and urinary excretion

Table 6

Average daily energy retention.

Treatment	Energy in feed	Energy in feces	Energy in urine	Energy retained	Energy retained
	Mcal	Mcal	Mcal	Mcal	%
1. Control	19.05	3.53	0.60	14.92	78.3
2. Zn	20.19	3.48	0.66	16.05	79.5
3. Cu	22.08	4.19	0.81	17.08	77.4
4. Zn + Cu	22.23	3.53	0.60	18.10	81.4
5. Mn	16.59	2.37	0.49	13.73	82.8
6. Zn + Mn	15.85	3.25	0.57	12.03	75.9
7. Cu + Mn	16.85	2.37	0.76	13.54	81.1
8. Zn + Cu + Mn	15.81	2.67	0.49	12.65	80.0
Overall average					79.5
Factor Level					
Zn Unsupplemented					79.8
Zn Supplemented					79.2
Cu Unsupplemented					79.1
Cu Supplemented					80.0
Mn Unsupplemented					79.1
Mn Supplemented					80.0
Standard error					0.9

of energy.

Concentration of Zinc, Copper and Manganese in Liver, Heart and Blood Samples

On the average, higher concentrations of zinc were found in liver than in kidney and heart tissue (Table 7).

The addition of zinc and copper to the rations did not have significant effects ($P<0.05$) on concentrations of zinc in liver tissue, although copper appeared to result in a slight decrease. The addition of manganese, and particularly in combination with zinc and copper, appeared to increase the concentration of zinc in liver tissue. This may not have been a real increase since the calves in Treatments 5 to 8 were tested at a later date than those in Treatment 2 to 4. However, one of the calves in Treatment 1 was included in the same experimental period as those in Treatments 5 to 8, and its copper concentration in the liver was approximately the same as that of its counterpart in the earlier period. This suggests that the addition of manganese did result in higher concentrations of zinc in liver tissue.

Supplemental zinc or copper did not affect the concentration of zinc in kidney tissue (Table 7). However, manganese alone, or in combination with zinc, or zinc and copper, appeared to increase kidney concentrations of zinc. As a result, there was a significant increase ($P<0.05$) associated with manganese supplementation.

Manganese, in combination with zinc, copper, or zinc and copper, appeared to increase the concentration of zinc in heart tissue, resulting in a highly significant increase ($P<0.01$) associated with supplemental manganese.

The addition of zinc, copper, or zinc and copper to the rations

Table 7

Concentrations of zinc in liver, kidney, heart and blood samples.

Treatment	Tissue[1]			Blood
	Liver	Kidney	Heart	
	ppm	ppm	ppm	ppm
1. Control	136	81	83	3.1
2. Zn	135	87	82	2.9
3. Cu	116	83	81	2.4
4. Zn + Cu	130	91	84	2.9
5. Mn	169	107	84	4.3
6. Zn + Mn	158	112	95	3.3
7. Cu + Mn	155	78	90	6.7
8. Zn + Cu + Mn	218	109	90	3.3
Factor Level				
Zn Unsupplemented	144	87	85	4.1
Zn Supplemented	160	100	88	3.1*
Cu Unsupplemented	150	97	86	3.4
Cu Supplemented	155	90	86	3.8
Mn Unsupplemented	129	85	83	2.8
Mn Supplemented	175*	102*	90**	4.4**
Standard error	11	4	1	0.3

1 On dry matter basis.
* Statistically significant ($P<0.05$).
** Statistically significant ($P<0.01$).

appeared to reduce the concentration of zinc in blood (Table 7), whereas manganese, and in particular manganese plus copper, appeared to increase the concentration of zinc in blood. When the factorial means were calculated (Table 7), there was a significant decrease ($P<0.05$) in blood concentration of zinc with the addition of zinc but a highly significant increase ($P<0.01$) with the addition of manganese to the ration.

The concentration of copper in liver tissue was much higher than in kidney, heart or blood samples (Table 8). The addition of zinc to the ration resulted in a marked reduction in the concentration of copper, whereas manganese resulted in some increase and copper resulted in a marked increase in the liver concentration of copper. When zinc was added with copper, there was some increase in the liver concentration of copper, but the highest levels were found when copper and manganese were added together. The addition of zinc, copper and manganese also resulted in a high liver concentration of copper. When factorial means were calculated (Table 8), zinc was associated with a significant decrease ($P<0.05$), copper was associated with a highly significant increase ($P<0.01$),and manganese was associated with a significant increase ($P<0.05$) in the liver concentration of copper. It appeared that zinc in the diet decreased the liver concentration of copper, that manganese partially offset the depressing effect of zinc, and that manganese increased the response to supplemental copper.

There were no appreciable effects on copper concentrations in kidney, heart or blood associated with the addition of the trace minerals to the rations (Table 8).

The inhibitory effect of zinc on liver concentration of copper

Table 8

Concentrations of copper in liver, kidney, heart and blood samples.

Treatment		Tissue[1]			Blood
		Liver	Kidney	Heart	
		ppm	ppm	ppm	ppm
1.	Control	144	14.3	16.7	0.48
2.	Zn	38	16.0	17.5	0.57
3.	Cu	271	15.9	18.6	0.58
4.	Zn + Cu	188	18.3	18.3	0.55
5.	Mn	184	16.7	17.4	0.53
6.	Zn + Mn	122	15.8	15.9	0.48
7.	Cu + Mn	357	17.5	18.1	0.51
8.	Zn + Cu + Mn	305	18.2	17.2	0.57
Factor	Level				
Zn	Unsupplemented	239	16.1	17.7	0.52
	Supplemented	163*	17.1	17.3	0.54
Cu	Unsupplemented	122	15.7	16.9	0.51
	Supplemented	280**	17.5	18.1	0.55
Mn	Unsupplemented	160	16.2	17.8	0.54
	Supplemented	242*	17.1	17.2	0.52
Standard error		21	0.7	0.4	0.02

1 On dry matter basis.
* Statistically significant ($P<0.05$).
** Statistically significant ($P<0.01$).

Table 9

Concentrations of manganese in liver, kidney and heart.

Treatment	Tissue[1]		
	Liver	Kidney	Heart
	ppm	ppm	ppm
1. Control	7.7	3.4	1.3
2. Zn	7.7	3.5	1.8
3. Cu	8.0	3.6	1.5
4. Zn + Cu	7.9	4.6	1.8
5. Mn	9.6	3.7	1.7
6. Zn + Mn	8.7	3.8	1.7
7. Cu + Mn	8.1	3.6	1.7
8. Zn + Cu + Mn	9.0	3.9	1.7
Factor Level			
Zn Unsupplemented	8.4	3.6	1.6
Zn Supplemented	8.3	4.0	1.7
Cu Unsupplemented	8.4	3.6	1.6
Cu Supplemented	8.3	3.9	1.7
Mn Unsupplemented	7.8	3.8	1.6
Mn Supplemented	8.9*	3.8	1.7
Standard error	0.3	0.2	0.1

[1] On dry matter basis.
* Statistically significant ($P < 0.05$).

agrees with the results of other research (Davis, 1958; McCall and Davis, 1961; Ritchie et al., 1963). The increase in liver concentration of copper associated with higher dietary levels of copper agrees with data reported by Dick (1954) and Gartner (1968).

The concentrationsof manganese in liver, kidney and heart tissue (Table 9) were much lower than those of zinc (Table 7) or copper (Table 8), and were too small to be measured in blood samples. The addition of zinc and copper to the ration had little effect on tissue concentrations of manganese, but the addition of manganese resulted in a small and significant increase ($P<0.05$) in the liver concentration of manganese.

Gastrointestinal Sites of Absorption and Endogenous Secretion

Zinc

When zinc was not added to the diet, there was a net loss of zinc, with 19 percent more found in the feces than was consumed in the feed (Table 10). This was associated with a large secretion into the reticulo-rumen and some net absorption found from the omasum, abomasum and large intestine. When zinc was added to the diet, there was 35 percent less zinc in the feces than was consumed, representing a net gain to body tissue. Supplemental zinc reduced net secretion into the reticulo-rumen, but did not appreciably alter net absorption found from the omasum, abomasum or large intestine.

The average net absorption of 35 percent of dietary zinc when supplemental zinc was added to the diet agrees fairly well with data reported by Feaster et al. (1954) and Miller and Cragle (1965).

In the present experiment, substantial amounts of zinc could

Table 10

Apparent net absorption (+) or secretion (-) of zinc
along the gastrointestinal tract at slaughter

(% of daily intake[1])

Segment	Zn		Cu		Mn		Standard error
	Unsupplemented	Supplemented	Unsupplemented	Supplemented	Unsupplemented	Supplemented	
Reticulo-rumen	-270[2] (-270)[3]	-65** (-65)**	-125 (-125)	-210 (-210)	-231 (-231)	-104 (-104)	41
Omasum	-77 (48)	-5 ** (30)	-21 (41)	-62** (37)	-64 (46)	-18** (32)	6
Abomasum	-39 (18)	25** (30)	11 (26)	-24** (22)	-15 (32)	1 (15)*	5
Small intestine	-33 (-4)	5* (-30)	-13 (-33)	-14 (-1)	-10 (-15)	-17 (-19)	8
Cecum	-37 (-3)	7** (-7)	-13 (-2)	-17 (-8)	-31 (-24)	1* (14)	9
Large intestine	-15 (13)	20** (13)	-1 (10)	6 (16)	0 (21)	5 (5)*	4
Feces	-19 (-7)	35** (14)	3 (3)	13 (4)	5 (4)	11 (3)	6

1 Each mean represents an average of eight animals.
2 Absorption or secretion accomplished up to a particular segment of the tract.
3 Absorption or secretion in a particular segment in relation to the previous segment.
* Statistically significant ($P<0.05$).
** Statistically significant ($P<0.01$).

have been supplied by drinking water which was conducted through galvanized pipes and drinking bowls. This effect was not measured, but Blackmon et al. (1967) indicated that galvanized pipes add appreciable amounts of zinc to the drinking water.

The addition of copper to the diet did not appear to affect net absorption of zinc appreciably (Table 10), since there was a net absorption of 13 and 3 percent zinc from the gastrointestinal tract in the presence or absence of copper, respectively. Supplemental copper appeared to increase secretion of zinc into the reticulo-rumen, decrease secretion into the small intestine and increase absorption found from the large intestine.

Supplementation of the diet with manganese (Table 10) did not appear to affect zinc absorption, since there was a similar net absorption of zinc found in the feces in the presence or absence of supplemental manganese. At the same time, manganese was associated with a decrease in net secretion of zinc into the reticulo-rumen, a decrease in net absorption found from the omasum, abomasum and large intestine, and an increase in net absorption found from the cecum.

There was net secretion of zinc into the reticulo-rumen with all animals in the experiment, which agees with reports by Miller and Cragle (1965) and Miller (1967).

Net absorption was found from the omasum and abomasum, ranging from 30 to 48 percent and 15 to 32 percent, respectively, of the zinc in the ingesta in each of those segments compared with that in the previous segment. Net secretion of zinc into the small intestine ranged from 1 to 30 percent, but was not significantly affected ($P<0.05$) by trace mineral supplementation. Net secretion of some zinc occurred in the cecum, except supplemental manganese was associated with net

absorption of zinc from the cecum. Net absorption of zinc was found from the large intestine, as compared with the previous segment, and was significantly reduced ($P<0.05$) by supplemental manganese.

Copper

Supplemental zinc in the diet was associated with a significant reduction ($P<0.01$) in total net absorption of copper (Table 11). This was associated with significantly less net absorption from the reticulo-rumen, increased net absorption from the omasum and net secretion in the feces. This agrees with general recognition of zinc as an inhibitor of copper absorption (Starcher, 1969).

Supplementation of the diet with copper resulted in a significant increase ($P<0.01$) in total net absorption of copper from 33 to 60 percent (Table 11). In the unsupplemented diets, there was net secretion in the reticulo-rumen, abomasum and feces; in the supplemented diets, there was net secretion only in the abomasum and net absorption was increased primarily from the reticulo-rumen and feces.

Supplementation of the diet with manganese resulted in a significant decrease ($P<0.01$) in total net absorption of copper from 53 to 40 percent (Table 11). In the presence of additional manganese, there appeared to be decreased absorption from the reticulo-rumen and large intestine, but increased absorption from the cecum.

A comparison of each segment of the gastrointestinal tract with the previous segment indicates that net secretion of copper occurred consistently only in the abomasum. When copper was added to the diet, there was a significant decrease ($P<0.05$) in net absorption of copper from the omasum.

Table 11

Apparent net absorption (+) or secretion (-) of copper along the gastrointestinal tract at slaughter

(% of daily intake[1])

Segment	Zn		Cu		Mn		Standard error
	Unsupplemented	Supplemented	Unsupplemented	Supplemented	Unsupplemented	Supplemented	
Reticulo-rumen	25[2] (25)[3]	4** (4)**	-15 (-15)	44** (44)**	19 (19)	9 (9)	4
Omasum	34 (11)	31 (21)	16 (25)	48 (7)*	40 (18)	24 (14)	3
Abomasum	8 (-29)	12** (-32)	-18 (-33)	34** (-29)	18 (-34)	3 (-27)	7
Small intestine	29 (15)	24 (13)	10 (18)	43 (11)	38 (18)	16 (11)	5
Cecum	41 (12)	35* (11)	27 (14)	49** (8)	42 (2)	34* (20)	2
Large intestine	46 (11)	45 (15)	32 (10)	58** (15)	54 (19)	38** (6)*	2
Feces	50 (4)	43** (-5)	33 (-5)	60** (5)	53 (-2)	40** (1)	1

1 Each mean represents an average of eight animals.
2 Absorption or secretion accomplished up to a particular segment of the tract.
3 Absorption or secretion in a particular segment in relation to the previous segment.
* Statistically significant ($P<0.05$).
** Statistically significant ($P<0.01$).

Manganese

Supplementation of the diet with zinc had no apparent effect on total net absorption of manganese (Table 12). The feces contained 85 and 81 percent as much manganese as was consumed daily in the absence or presence of supplemental zinc. Although net absorption of manganese was significantly higher up to the omasum ($P<0.01$), abomasum ($P<0.05$) and large intestine ($P<0.01$), there was a marked increase in net secretion of manganese in the small intestine and feces associated with supplemental zinc.

Supplemental copper resulted in a significant decrease ($P<0.01$) in net absorption of manganese in the omasum, but had no apparent effects on the rest of the gastrointestinal tract.

The addition of manganese to the diet had a decreasing effect on total net absorption of manganese (Table 12). However, supplemental manganese was associated with increased absorption from the reticulo-rumen and omasum, reduced absorption from the abomasum and large intestine, and decreased secretion into the cecum.

There was net secretion of manganese into the small intestine and cecum, and net absorption of manganese from the large intestine. Supplemental manganese significantly decreased net secretion ($P<0.05$) of manganese into the cecum and significantly decreased ($P<0.01$) net absorption of manganese from the large intestine.

Dry Matter Distribution in Segments
of the Gastrointestinal Tract at Slaughter

The digesta in the reticulo-rumen accounted for 81.7 to 84.7 percent of the total dry matter in the gastrointestinal tract (Table 13). The percentage of dry matter contained in the remaining segments

Table 12

Apparent net absorption (+) or secretion (-) of manganese along the gastrointestinal tract at slaughter

(% of daily intake[1])

Segment	Zn		Cu		Mn		Standard error
	Unsupplemented	Supplemented	Unsupplemented	Supplemented	Unsupplemented	Supplemented	
Reticulo-rumen	-3[2] (-3)[3]	12 (12)	7 (7)	2 (2)	-5 (-5)	15 (15)	7
Omasum	-5 (-3)	19** (8)	16 (8)	-2** (-3)	-9 (-5)	23** (10)	5
Abomasum	-7 (-8)	50* (37)	38 (26)	5 (3)	19 (30)	25 (-1)	14
Small intestine	5 (-10)	19 (-52)	14 (-39)	10 (-24)	8 (-28)	16 (-34)	5
Cecum	-22 (-28)	-1 (-26)	-7 (-26)	-15 (-28)	-34 (-48)	12* (-6)*	11
Large intestine	-3 (11)	21** (22)*	8 (16)	9 (17)	4 (30)	14 (2)**	5
Feces	15 (16)	19 (-6)	19 (5)	16 (4)	21 (14)	13 (-5)	7

1 Each mean represents an average of eight animals.
2 Absorption or secretion accomplished up to a particular segment of the tract.
3 Absorption or secretion in a particular segment in relation to the previous segment.
* Statistically significant ($P<0.05$).
** Statistically significant ($P<0.01$).

Table 13

Dry matter in segments of the gastrointestinal tract at slaughter

(% of total[1])

Segment	Zn		Cu		Mn		Standard error
	Supplemented	Unsupplemented	Supplemented	Unsupplemented	Supplemented	Unsupplemented	
Reticulo-rumen	84.2	82.7	84.7	82.3	81.7	85.3	1.5
Omasum	2.5	3.8	2.8	3.5	3.7	2.6	0.5
Abomasum	4.6	4.8	4.3	5.1	5.2	4.2	0.4
Small intestine	4.4	4.2	3.7	4.9	4.4	4.2	0.5
Cecum	1.6	1.8	1.9	1.4	1.6	1.8	0.2
Large intestine	2.7	2.7	2.5	2.8	3.5	1.8	0.5

[1] Each mean represents an average of eight animals.

ranged from 2.5 to 3.8, 4.2 to 5.2, 3.7 to 4.9, 1.4 to 1.9 and 1.8 to 3.5 for the omasum, abomasum, small intestine, cecum and large intestine, respectively.

No significant ($P<0.05$) differences were detected between treatments.

Excretion of Zinc, Copper and Manganese

In the absence of supplemental zinc, more zinc was excreted in the feces than was consumed in the diet (Table 14). This might be attributed to a substantial contribution of zinc from the drinking water. The addition of zinc to the diet (Treatment 2), zinc and copper (Treatment 4), zinc and manganese (Treatment 6) or zinc, copper and manganese (Treatment 8) resulted in less zinc excreted in the feces than was consumed in the diet, or apparent net retention of zinc. Overall, supplementation with zinc significantly reduced ($P<0.01$) fecal content of zinc, and copper and manganese had no significant effect ($P<0.05$) on fecal content of zinc.

All three trace minerals had significant effects ($P<0.01$) on fecal excretion of copper (Table 15), as indicated by the factorial means. Zinc and manganese increased and copper decreased fecal excretion of copper. The significant interactions indicated that supplemental copper reduced ($P<0.01$) the increase in copper excretion caused by supplemental zinc (Treatment 4) and manganese (Treatment 7).

The fecal excretion of manganese (Table 14) was not significantly affected ($P<0.05$) by any of the supplemental trace minerals, nor were there any significant interactions ($P<0.05$) between the trace minerals.

Urinary excretion of the trace minerals (Table 15), as a percentage of dietary consumption, was small in comparison with fecal

Table 14

Excretion of zinc, copper and manganese in feces

(% of daily intake)

Treatment		Zinc	Copper	Manganese
1.	Control	121.6	55.8	80.5
2.	Zn	67.0	57.6	77.5
3.	Cu	130.9	30.2	81.4
4.	Zn + Cu	59.4	46.2	75.7
5.	Mn	110.8	80.8	91.0
6.	Zn + Mn	88.4	75.5	74.8
7.	Cu + Mn	111.3	33.1	85.1
8.	Zn + Cu + Mn	44.4	49.8	95.4
Factor	Level			
Zn	Unsupplemented	118.7	50.0	84.5
	Supplemented	64.8**	57.2**	80.8
Cu	Unsupplemented	97.0	67.4	81.0
	Supplemented	86.5	39.8**	84.4
Mn	Unsupplemented	94.8	47.4	78.8
	Supplemented	88.8	59.8**	86.6
Standard error		5.7	1.1	6.8

** Statistically significant ($P < 0.01$).

Table 15

Excretion of zinc, copper and manganese in urine.

(% of daily intake)

Treatment		Zinc	Copper	Manganese
1. Control		2.59	10.67	0.45
2. Zn		0.76	10.26	0.39
3. Cu		2.25	16.49	0.45
4. Zn + Cu		0.45	2.16	0.33
5. Mn		0.96	1.41	0.15
6. Zn + Mn		0.85	0.95	0.18
7. Cu + Mn		1.63	0.84	0.28
8. Zn + Cu + Mn		1.47	1.11	0.22
Factor	Level			
Zn	Unsupplemented	1.86	7.36	0.34
	Supplemented	0.89*	3.62* *	0.29
Cu	Unsupplemented	1.29	5.83	0.30
	Supplemented	1.45	5.15	0.33
Mn	Unsupplemented	1.52	9.90	0.41
	Supplemented	1.23	1.08**	0.21**
Standard error		0.27	0.43	0.03

* Statistically significant ($P < 0.05$).
** Statistically significant ($P < 0.01$).

excretion (Table 14). This indicates that the major pathway of excretion of all three trace minerals was in the feces.

Urinary excretion of zinc was significantly reduced ($P<0.05$) by the addition of zinc to the diet, which agrees with the report of Miller *et al.* (1970), but was not significantly affected ($P<0.05$) by additional copper or manganese, and none of the interactions was significant ($P<0.05$).

Miller *et al.* (1966a) reported increased urinary excretion of ^{65}Zn in calves fed a zinc-deficient diet (6 ppm Zn), but no effect of dietary zinc was noted on urinary excretion of zinc from normal animals. In the present experiment the unsupplemented diets contained 40 ppm of zinc, which is close to the value of 50 ppm of zinc suggested as a practical level for calves (Thompson, 1970). Therefore, these diets may have been borderline in their content of zinc, and not sufficiently deficient to produce any biological symptoms of a deficiency.

Urinary excretion of copper was significantly reduced ($P<0.01$) by supplemental zinc and manganese, but not by copper, as indicated by the factorial means (Table 15). However, zinc appeared to have this effect only when in combination with copper (Treatment 4 vs Treatment 2), manganese (Treatment 6) or copper and manganese (Treatment 8). Manganese appeared to reduce copper excretion when alone or in any combination with zinc or copper.

Urinary excretion of manganese (Table 15) was low with all treatments. It was significantly reduced ($P<0.01$) by manganese supplementation of the diet, but was not significantly affected ($P<0.05$) by zinc or copper. None of the interactions was significant ($P<0.05$).

General Discussion

Differences in average daily feed consumption, daily gain, and feed consumed per unit gain obtained in this experiment were relatively small. Calves fed the basal ration supplemented with zinc and manganese had the lowest rate of gain and average daily feed consumption, but feed conversion was comparable to that of calves fed the basal ration with the zinc or manganese supplement. Apparent digestibility of dry matter, nitrogen and gross energy was also lowest in calves fed the basal ration supplemented with zinc and manganese.

Since calves fed the ration supplemented with zinc or manganese had daily feed consumption, rate of gain, and apparent digestion coefficients comparable to those of calves not fed these supplements, this would suggest that there was some interrelationship between zinc and manganese affecting feed utilization by calves. However, only the differences in digestibility of nitrogen and gross energy were significant. This could be partially affected by the lower digestibility of dry matter in this ration resulting in greater fecal excretion, and lower retention of nitrogen and gross energy.

It was indicated by factorial means that there were no significant differences that could be attributed to the addition of the trace minerals. Consequenlty, the experiment showed that the addition of zinc, copper and manganese in excess to a ration composed primarily of barley, and which contained 40 ppm zinc, 6 ppm copper and 12 ppm manganese, did not improve the performance of fattening bull calves during a feeding period of 70 days.

High dietary zinc did not affect its concentration in the liver, kidney and heart tissues. Miller et al. (1970) reported a significant increase in the zinc concentration in liver when a diet containing 33 ppm zinc was increased in zinc content to 233 ppm, but no effect on zinc concentrations in kidney and heart tissues. The lack of increase in the zinc concentration in the liver in this experiment could be caused by the relatively low, 2-fold, increase in zinc in the diet as compared with the 7-fold increase used by Miller et al. (1970). High dietary copper levels did not affect the concentration of zinc in the liver, kidney and heart tissues. However, dietary manganese was associated with a significant increase in zinc concentration of the liver, kidney and heart tissues.

Supplemental zinc significantly decreased its excretion in the feces and urine, and was associated with increased net absorption. Net secretion of zinc appeared in the reticulo-rumen and was significantly decreased by the addition of zinc in the diet. No additional effect of dietary zinc was apparent in other segments of the gastrointestinal tract. Net secretion of zinc was also found in the small intestine and cecum. Net secretion in the reticulo-rumen and first section of the small intestine, and net absorption in the last two sections of the small intestine and the other segments of the gastrointestinal tract was reported by Miller et al. (1967). Yang and Thomas (1965) indicated that there was a large secretion in the upper small intestine of a number of other nutrients, including dry matter, ash, calcium, phosphorus, sodium and water. In the present experiment the entire small intestine was analysed as a single segment, and there was an indication of net secretion with all treatments. Net absorption of zinc took place in the

omasum and abomasum.

Bremner (1970) found zinc solubility in the rumen of sheep to be approximately 10 percent, although over 50 percent of the zinc in the diet was water-soluble. Large proportions of the zinc in the rumen may have been converted into an insoluble complex which could explain the relatively low zinc absorption in this experiment; when zinc was added in the diet, higher proportions of soluble zinc could have been available, resulting in the higher rate of net zinc absorption that was obtained.

There were no appreciable differences between treatments in respect to the fecal and urinary excretion and the net absorption of zinc due to dietary copper and manganese although significant differences in some segments of the gastrointestinal tract were apparent.

The significant decrease in blood uptake of zinc, associated with zinc addition of the diet, was not in accordance with the net zinc absorption. However, the significance of this is doubtful, since calves in treatment 7 had approximately a 2-fold higher zinc concentration in the blood than the rest of the calves, and this contributed to the higher overall mean. Since the diet in treatment 7 was supplemented with copper and manganese, and the interaction of these minerals was not significant, the cause of the higher level of blood zinc in this treatment cannot be explained.

Higher dietary manganese significantly increased the concentration of zinc in blood, and was associated with a significant increase in concentrations of zinc in the liver, kidney and heart tissues, and with higher absorption and slightly lower urinary excretion of zinc.

In this experiment it appeared that there was no deficiency in any treatment, and that high dietary manganese together with high

dietary zinc could have harmful affects on cattle, since accumulation of zinc in the tissue would take place.

Supplemental trace minerals appeared to have little effect on concentrations of copper in kidney and heart tissue, and on uptake of copper by blood. Liver tissue reflected the trace mineral contents in the diet. Higher dietary zinc significantly decreased the copper concentration in liver. The inhibitory effect of zinc on liver concentrations of copper agrees with the results of other research (Davis, 1958; McCall and Davis, 1961; Ritchie et al., 1963). The significant increase in liver concentration of copper, associated with its higher dietary levels, agrees with reports of Dick (1954) and Gartner et al., (1968). The significant increase in liver concentration of copper was also associated with higher dietary manganese.

More copper was excreted in the feces when zinc was supplemented in the diet, indicating lower net absorption. McCall and Davis (1961) suggested that formation of copper complexes with zinc, protein and other nutrients may affect copper absorption from the gastrointestinal tract. The results of this experiment indicated that possible formation of copper-zinc complexes took place in the reticulo-rumen, since a significant decrease of net copper absorption, associated with higher dietary zinc, appeared in this segment and was not appreciably affected in the other segments of the gastrointestinal tract. The lower rate of fecal excretion of copper was associated with higher dietary levels. The influence of copper on its absorption took place in the reticulo-rumen and omasum. When manganese was supplemented in the diet, the secretion of copper in feces was increased, causing a lower rate of net absorption. Differences in absorption of copper along the

gastrointestinal tract associated with manganese in the diet were apparent after the small intestine.

In general, there was net secretion of copper into the abomasum in all treatments. Net absorption appeared in the rest of the segments except in the reticulo-rumen, where net secretion was associated with low dietary copper.

In the previous discussion high dietary manganese was associated with increased concentration of copper in the liver and decreased absorption from the gastrointestinal tract. However, dietary manganese was also associated with a 10-fold decrease in urinary excretion of copper. This would suggest that dietary manganese increased the incidence of binding sites of copper in the liver tissue, thereby increasing incorporation of copper into the liver and decreasing its elimination in the urine.

There was no evidence of copper deficiency, but high levels of supplemental zinc in a diet containing a low level of copper could create copper deficiency. The very low concentration of copper in livers of calves fed low copper and high zinc in the diet (Treatment 2), indicated that deficiency could develop if the experiment continued for a longer period. High dietary zinc might be useful in prevention of copper toxicity in cattle.

There were very small and nonsignificant differences in the fecal excretion and absorption of manganese between treatments, associated with the addition of zinc, copper and manganese in the diet. Bremner (1970) indicated that only about 10 percent of manganese in the rumen of sheep was in a soluble form, although over 50 percent manganese in the diet was soluble in water. The results of the present experiment agree

with those findings, since a very low rate of absorption of manganese appeared in the reticulo-rumen, and total net absorption was also relatively low. Relatively high secretion occurred in the cecum in all treatments. Lower total absorption of manganese associated with the higher dietary level indicated that manganese is preferentially absorbed when the dietary intake is low which agrees with Howes and Dyer (1971).

Higher dietary zinc and copper did not affect urinary excretion, or concentrations of manganese in the liver, kidney and heart tissues. Higher dietary manganese significantly decreased its excretion in the urine and increased its incorporation into the liver, but not into the kidney and heart. Howes and Dyer (1971) reported a similar action of dietary manganese upon urinary excretion and liver concentration of manganese in calves at seven days of age.

In this experiment, it appeared that an all-barley ration could be fed to fattening bull-calves without the trace mineral supplements. The formulation of the ration should be balanced for all trace minerals; copper should be increased if there is a high content of zinc in the diet, and both zinc and copper should be kept low if there is a high content of manganese in the diet.

Summary and Conclusions

Calves fed the basal ration supplemented with zinc and manganese had the lowest rate of gain, average daily feed consumption, and apparent digestibility of dry matter, nitrogen and gross energy. This suggested that some interrelationship between zinc and manganese affected feed utilization by calves.

Higher dietary manganese increased the concentration of zinc in blood, and was associated with increased concentrations of zinc in the liver, kidney and heart tissues, and with higher absorption and slightly lower urinary excretion of zinc.

The liver concentration of copper reflected its dietary intake but was decreased by supplemental zinc. Dietary manganese was associated with increased concentration in the liver, decreased absorption from the gastrointestinal tract and a 10-fold decrease in urinary excretion of copper. This suggested that dietary manganese increased the incidence of binding sites of copper in the liver tissue.

Supplemental manganese decreased its excretion in the urine and increased its incorporation into the liver, but not into the kidney and heart. Manganese was preferrentially absorbed when its dietary intake was low.

On the basis of the results obtained in this study, it is suggested that an all-barley ration could be fed to fattening bull-calves without the trace mineral supplements during a feeding period of 70 days.

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Appendix Table 1

Dry matter of liver, kidney and heart.

Treatment		Tissue		
		Liver	Kidney	Heart
		%	%	%
1.	Control	27.5	25.5	21.1
2.	Zn	25.4	30.3	22.9
3.	Cu	26.6	25.4	22.2
4.	Zn + Cu	26.0	27.9	21.3
5.	Mn	25.9	21.7	20.2
6.	Zn + Mn	27.2	23.4	20.0
7.	Cu + Mn	27.1	20.7	19.7
8.	Zn + Cu + Mn	28.0	24.1	20.3
Factor	Level			
Zn	Unsupplemented	26.8	23.3	20.8
	Supplemented	26.7	26.4	21.1
Cu	Unsupplemented	26.5	25.2	21.0
	Supplemented	26.9	24.5	20.9
Mn	Unsupplemented	26.4	27.3	21.8
	Supplemented	27.0	22.5	20.0
Standard error		1.0	1.2	0.2

Table 2

Mean squares obtained by analysis of variance of daily feed and gain, digestibility, excretion of trace minerals and concentration of trace minerals in tissues

Variables	Mean squares								Total sum of squares
	Mn	Cu	MnxCu	Zn	MnxZn	CuxZn	MnxCuxZn	Error	
d.f. →	1	1	1	1	1	1	1	8	15
Average daily feed on metabolic wt. basis	3.06	0.81	0.09	0.02	0.30	0.16	1.96	1.00	14.39
Average daily gain	0.31	0.02	0.00	0.00	0.02	0.00	0.03	0.06	0.87
Average feed/kg gain	0.36	0.04	0.18	0.26	0.09	0.14	0.02	0.14	2.22
Apparent dry matter digestion	3.17	7.02	0.46	3.67	30.53	3.82	4.77	6.60	106.23
Apparent nitrogen digestion	8.90	0.12	0.49	16.95	60.41*	3.72	6.88	9.06	169.97
Apparent energy digestion	5.97	5.63	1.43	4.36	44.66*	6.75	1.11	6.86	124.85
Percent nitrogen retention	116.53	0.50	50.91	60.76	107.23	56.78	7.00	92.99	1143.59
Percent energy retention	3.06	3.24	0.30	1.69	42.90*	17.64	2.72	6.54	123.88
Liver zinc concentration	8441.02*	110.78	1283.43	1080.77	367.68	1973.58	919.61	938.04	21681.16
Kidney zinc concentration	1036.84*	160.02	370.56	605.16	125.44	197.40	138.06	118.63	3582.50
Heart zinc concentration	199.52**	0.00	0.01	41.28	19.28	15.41	52.20	11.44	419.55
Blood zinc concentration	10.24**	0.90	2.40	4.00*	6.25*	0.90	2.40	0.61	31.96
Liver copper concentration	26781.32*	99761.22**	1604.00	22876.56*	1402.50	277.22	40.32	3444.43	180298.60
Kidney copper concentration	3.33	12.43	0.14	3.90	4.95	1.38	0.23	3.41	53.68
Heart copper concentration	1.69	5.29	0.12	0.90	2.25	0.04	0.72	1.11	19.85
Blood copper concentration	0.002	0.005	0.000	0.002	0.001	0.000	0.013	0.005	0.061
Liver manganese concentration	4.15*	0.11	0.74	0.03	0.00	0.66	0.95	0.66	11.92
Kidney manganese concentration	0.00	0.39	0.48	0.62	0.13	0.32	0.10	0.28	4.27
Heart manganese concentration	0.07	0.00	0.00	0.12	0.14	0.00	0.01	0.09	1.08
Percent fecal Zn excretion	145.20	434.72	515.29	11599.29**	336.72	939.42	190.44	260.96	16248.76
Percent fecal Cu excretion	610.07**	3050.16**	330.08**	211.67**	10.23	326.11**	15.64	9.80	4632.38
Percent fecal Mn excretion	241.77	47.27	60.44	54.36	2.31	140.50	212.80	369.23	3713.25
Percent urinary Zn excretion	0.33	0.10	0.95	3.79*	2.81	0.00	0.00	0.58	12.60
Percent urinary Cu excretion	311.17**	1.82	0.87	55.80**	52.93**	43.49**	53.73**	1.51	531.90
Percent urinary Mn excretion	0.16**	0.00	0.01	0.01	0.01	0.01	0.00	0.01	0.30

*P< 0.05; **P< 0.01

Table 3

Mean squares obtained by analysis of variance of apparent net absorption or secretion of trace minerals along the gastrointestinal tract at slaughter

Variables	Mean squares								Total sum of squares
	Mn	Cu	MnxCu	Zn	MnxZn	CuxZn	MnxCuxZn	Error	
d.f.→	1	1	1	1	1	1	1	8	15
Zinc									
Reticulo-rumen	63650	29196	52858	168633**	8141	41038	64140	13496	535629
Omasum	8468**	6653**	5081**	20746**	16743**	2683*	6858**	338	69935
Abomasum	950	4871**	3392**	16184**	9834**	718	2114*	219	39812
Small intestine	178	3	1	5775*	6102*	1274	608	552	18355
Cecum	4025**	54	1344	7690**	7029*	1006	232	634	26455
Large intestine	128	207	6	5036**	4973**	387	706	146	12612
Feces	145	435	514	11602**	337	937	190	261	16249
Copper									
Reticulo-rumen	390	13631**	431	1661**	2048**	5	1243*	140	20527
Omasum	1008*	4128**	946*	46	189	915*	518*	91	8480
Abomasum	798	9851**	2783*	60	371	1314	281	364	18372
Small intestine	1892	4356	2304**	100	441	2256**	90	198	13022
Cecum	210*	2070**	992**	144	361**	1156**	169*	28	5328
Large intestine	961**	2162**	576**	1	210*	1296**	182*	24	5578
Feces	610**	3050**	330**	212**	10	326**	16	10	4632
Manganese									
Reticulo-rumen	1580	105	2	885	248	116	352	424	6676
Omasum	4096**	1332*	462	2401**	1225*	132	506	185	11634
Abomasum	144	4422	42	13225*	676	702	2	1613	32115
Small intestine	281	77	23	856	28	1040	18	224	4116
Cecum	8510*	248	18	1785	95	138	1508	956	19974
Large intestine	400	4	420	2209	30	462	361	169	5236
Feces	242	47	60	54	2	140	213	369	3713

*P<0.05; **P<0.01

Table 4

Mean squares obtained by analysis of variance of absorption or secretion in a particular segment in relation to the previous segment of the gastrointestinal tract

Variables	Mean squares								Total sum of squares
	Mn	Cu	MnxCu	Zn	MnxZn	CuxZn	MnxCuxZn	Error	
d.f.→	1	1	1	1	1	1	1	8	15
Zinc									
Omasum	724	92	333	1236	4085**	567	72	235	8946
Abomasum	1163*	96	107	497	33	149	211	164	3568
Small intestine	86	3937	2790	2664	132	605	135	953	17972
Cecum	6108	134	1352	61	71	311	393	1636	21517
Large intestine	1005*	157	843*	2	1	171	203	123	3367
Feces	10	8	384	1800*	2690**	2429**	2127*	191	10980
Copper									
Omasum	94	1269*	54	446	818	386	66	208	4795
Abomasum	182	66	975	38	1081	1745	111	803	10620
Small intestine	193	210	109	16	376	1807	244	505	6995
Cecum	1965	13	145	42	56	2	1	638	7327
Large intestine	699*	108	212	74	0	22	0	71	1684
Feces	46	400	42	319	216	648*	81	111	2640
Manganese									
Omasum	924	521	135	494	452	148	1382*	225	5854
Abomasum	4020	2273	723	7946	338	526	1807	3330	44274
Small intestine	139	954	275	6991	3511	870	50	2801	35200
Cecum	7171*	18	79	22	79	620	1748	749	15730
Large intestine	3108*	1	77	456*	91	56	286	83	4741
Feces	1472	9	32	1989	2	280	47	702	9446

*P<0.05; **P<0.01